REMARKS

Claim 32 has been amended to limit the organisms from which the extract is obtained for use in the method to plants, insects, mammals, fish, reptiles and birds. Support for plants is found in claim 33 and for mammals is found in claim 35. Support for fish, birds and reptiles is found on page 12, beginning at line 35. Support for insects is found on page 12, at line 18. Claim 34 has been amended to limit the organisms to an insect, a mammal or bird, supported as set forth above. In addition, new claim 67 is proposed which is supported by the specification, for example, on page 2, at lines 12-20. Thus, no new matter has been added and entry of the amendment is respectfully requested.

Thus, claims 32-37, 39-41, 49 and 66-67 are pending in this application. Claims 33, 34, 39 and 40 are withdrawn from consideration as being directed to non-elected species. Claims 32, 35-37, 49 and 66 were examined and stand rejected.

Request for Withdrawal of Finality

The present rejection was made final based on the assertion that the new grounds for rejection were necessitated by amendment. Applicants believe this is not the case. While claims 32 and 41 were amended in the previous response, this amendment did not necessitate the new grounds for rejection.

Thus, the unamended claim 32 was already distinguished over Claycomb and Lanson by virtue of its preamble which was directed to a method of detecting post-transcriptional gene silencing of a target gene in an organism. The amendment, which required that the extract be prepared from an organism in which it was suspected that PTGS is occurring, simply made explicit what was already inherent in the claim by virtue of the preamble. It is well established that a

preamble is a claim limitation if it imposes limitations on the body of the claim. This was verified in *Corning Glass Works v. Sumitomo Electric U.S.A., Inc.*, 868 F2d 1251, 9 USPQ2d 1962 (Fed. Cir. 1989). In this case, the preamble stated that the claim was directed to a waveguide and the body of the claim enumerated certain structural features. These structural features appeared in the prior art, but not in the context of the waveguide. The Court held that the requirement in the preamble that the claim was directed to a waveguide imposed other structural limitations on the subject matter. Similarly, here, because the preamble stated that the claim was directed to a method of detecting PTGS of a target gene in an organism, this imposed the requirement in the actual method that the extract be prepared from an organism in which it was suspected that PTGS is occurring.

The amendment to clarify that the determination was of the presence, as opposed to the absence, of short RNA molecules, was not necessary to distinguish Crooke, but was simply for clarification so that no claim could be made that there was no infringement if no SRMs were actually detected. As noted in the previous response, Crooke teaches detecting the presence or absence of much shorter cleavage products.

Finally, the third step required by the claim did not appear anywhere in Crooke.

While claim 41 was also clarified, this clarification was not necessary to distinguish from Crooke for the reasons set forth to the response to this rejection.

Since the amendments made to the claims were focused on clarity as opposed to distinction from Crooke and not necessary to make this distinction, the amendment to the claims did not necessitate this new basis for rejection and applicants respectfully request that the finality of the rejection be withdrawn.

In any event, should the Office, for some reason, maintain its position that the finality of the rejection is proper, entry of the proposed amendments is believed justified as placing the claims in a better position for allowance or appeal. The amendment clearly obviates the anticipation rejection over Lee and the obviousness rejection over Lee combined with Schena.

Therefore, the Examiner is respectfully requested to exercise her discretion to enter the amendment to simplify any issues on appeal.

Inventorship

It is greatly appreciated that the Examiner has confirmed that the declaration on file is sufficient to create the presumption that the named inventors are the only inventors in this case and that no additional declaration refuting inventorship by Mr. L. Scott is necessary.

Priority

It is appreciated that the Examiner has confirmed that claims 32, 35-37, 49 and 66 are entitled to the 10/27/1999 priority date. The Examiner has also affirmed that claim 41 is entitled to a priority date of 01/26/2000. It remains unclear on the existing record why claim 41 is not accorded the 10/27/1999 priority date. However, this is academic with respect to the prior art cited in the present Office action.

Withdrawal of the 35 U.S.C. § 102(e) Rejection

Applicants are gratified that the § 102(e) rejection of claims as being anticipated by Crooke, *et al.*, has been withdrawn.

Election of Species vs. Restriction Requirement – Clarification Requested

In the prior Office action and in the outstanding Office action, claims 33, 34, 39 and 40 are said to be withdrawn from consideration as being drawn to non-elected subject matter. As pointed out in the previous response filed on 9 May 2008, bottom of page 4 to the top of page 5, applicants have confirmed that these claims were all included in Group I of the Restriction Requirement. Applicants have elected the species – mammals – for purposes of examination, with the understanding that should a linking claim be allowed, other species to which the linking claim is generic would be examined. Accordingly, retention of these claims is believed proper.

CLAIM REJECTIONS

1. The Rejection of Claims 32, 36, 37, 49, and 66 as Anticipated by Lee, *et al.* (1993, *Cell* 75:843-854, item 7 on 03/11/2008 IDS)

Claims 35 and 41 are free of this rejection.

According to the Office, Lee, *et al.,* teach the identification of two RNAs that were suspected of regulating *lin-14* gene expression in the nematode, *C. elegans*, by an antisense mechanism, detection of the *lin-4S* (about 22 nt) and *lin-4L* (sense and antisense sequences connected by a 7-nucleotide linker) transcripts believed to play a role in post-transcriptional silencing of the *lin-14* target gene. The detected transcripts were shown to have sequence similarity with respect to the 3′-UTR of the *lin-14* target gene.

The 3′-UTR does not qualify as a gene according to the definition of that term in the specification (page 4, lines 30-32) since it is not translated. However, to expedite prosecution, all claims are amended so that nematodes are not included within their scope. As Lee, *et al.*, only

disclose the *lin-4S* and *lin-4L* transcripts, and these transcripts are only disclosed in nematodes, this ground for rejection as to all claims of record (including withdrawn claims 33, 34, 39 and 40) may be withdrawn.

2. The Rejection of Claims 32, 33, 35, 36, and 66 as being Anticipated by Noonberg, *et al.*, (1997, U.S. Patent 5,624,803)

The characterization by the Office of what the Noonberg document teaches is neither confirmed nor contested. What is contested is the applicability of Noonberg as a reference against the present claims because nowhere in Noonberg is there any disclosure or suggestion that a nucleic acid extract should be prepared from an organism "in which organism it is suspected that PTGS is occurring." Rather, Noonberg, *e.g.*, in figures 5-7 referenced by the Examiner, show the detection of the deliberately generated antisense oligonucleotides in the "U6ON" transcript by Northern blot. This is not a method to detect PTGS in an organism in which it is suspected that PTGS is occurring. It is only to show that the transcript is being produced from the "oligonucleotides generator" that Noonberg, *et al.,* have intentionally introduced into the eukaryotic cells*. The detection of such transcript, in and of itself, is no indication whatsoever that post-transcriptional gene silencing is occurring. The organisms have been deliberately engineered to produce these transcripts.

The Office is again reminded, as was stated in the previous response and hereinabove, that the preamble to the claim is considered a restriction if it affects what is actually being done. (*Corning Glass Works v. Sumitomo Electric U.S.A., Inc., supra.*) Here, as in that case, the preamble

* See the description of figure 5: "FIG. 5 is a half-tone reproduction of Northern blots which demonstrate the production and nuclear localization of the U6ON transcript." Likewise, the description of figure 6: "FIG. 6 is a graph and a half-tone reproduction of a Northern blot which demonstrates the kinetics of U6ON expression." Likewise for figure 7: "FIG. 7 is a graph and a half-tone reproduction of a Northern blot which demonstrates the intracellular stabilities of the chimeric gene and the U6ON transcript."

which is directed to a method of "detecting post-transcriptional gene silencing of a target gene in an organism" clearly disqualifies Noonberg because no method of detecting post-transcriptional gene silencing by carrying out the intended steps is ever described. As is pointed out below, according to the method of Noonberg, any PTGS is detected by various other tests, since any SRMs are intentionally generated and their presence or absence gives no indication whatsoever as to whether any silencing is taking place.

In order to show that post-transcriptional gene silencing is in fact occurring, Noonberg, *et al.,* have to turn to secondary indicia – protein knockdown (see, *e.g.*, figure 11 – "a graph representing the effects of the U6ON and U6CTcon oligonucleotide generators on CAT ACTIVITY"), mRNA (see, *e.g.*, figure 12 – "Northern blots, which demonstrate the effects of the U6ON generator on endogenous HER2 mRNA"), etc., to determine whether or not the U6ON transcript, intentionally being produced from their oligonucleotides generator, is actually performing any function (whether as a triplex forming agent, as a ribozyme or as straight antisense molecule). Nowhere in the Noonberg disclosure is the mere detection of the U6ON transcript used as a direct indication that PTGS is occurring. It would have been nonsensical for Noonberg to have done so as those transcripts were being intentionally produced and may or may not have had any PTGS inducing effect.

By contrast, per the present invention, as disclosed and claimed, the mere detection of the SRMs which meet the definition of our claims is the necessary and sufficient indication that post-transcriptional gene silencing must be occurring, and, that by determining to which genes the detected SRMs bear sequence identity or similarity, it is in addition possible to determine which genes are the target genes that are being silenced. Thus, not only are the purposes of the detecting

that is undertaken per Noonberg and per the present invention completely different, the methods themselves are different because the method of the invention employs organisms in which it is suspected that PTGS is taking place whereas the method of Noonberg, *et al.,* uses organisms that have been modified to contain U6ON. The Noonberg method, by intentionally introducing short transcripts into the material to be examined, could potentially obscure the ability to use the present invention to achieve the goals to which applicants' claims are directed.[†]

Therefore, respectfully, Noonberg, *et al.*, does not anticipate and this ground for rejection may be withdrawn.

3. The Rejection of Claims 32, 35, 36, 37, 49 and 66 as being Unpatentable over Lee, *et al., supra*

Claim 41 is free of this rejection.

The thesis of the obviousness rejection is that while Lee, *et al.*, "do not explicitly teach the detection of PTGS in a mammal" by means of detecting SRMs, (which is what Lee, *et al.*, is asserted to have done in the worm, *C. elegans*), the mention by Lee, *et al.,* of the involvement of 3′UTR, which is known to be present in mammalian genes and are thought "to contain developmentally significant posttranscriptional regulatory sequences in their 3′UTR" suggests that "the possibility exists that such mammalian genes may be regulated by small RNAs much like the case of *lin-4* and *lin-14*" (page 851, first paragraph).

[†] At column 27, lines 55-56 of Noonberg, it is disclosed that: "U6ON could not be detected to any significant extent in the cytoplasmic fraction." This is in contrast to the occurrence of SRMs generated during PTGS primarily in the cytoplasm.

The Office asserts that "One of ordinary skill would immediately recognize the small RNA mediated PTGS in *C. elegans* as a likely means for PTGS in other organisms including mammals because Lee, *et al.,* explicitly suggest so." The Office, from this, goes on to assert that

> Those of skill in the art, biologists, are motivated to identify the conservation of biological functions among organisms and, as such, would be motivated to detect PTGS in mouse to evaluate the conservation of the small RNA-mediated regulation taught be Lee, *et al.* Therefore, the claims would have been obvious at the time of the instant invention.

All of this is contradicted by the published description of the history of gene silencing.

The problem with this thesis is that Lee, *et al.*, (a group led by Victor Ambros) and their collaborators (a group led by Gary Ruvkun) have since, themselves, commented directly on the obviousness or otherwise that their unique identification of a very peculiar RNA transcript in *C. elegans* would be expected to be a motif that would be recapitulated for any other genes even in nematodes, let alone in organisms as divergent as plants or mammals, and their conclusions do not match those of the Office.

Enclosed herewith is a publication from 2004 by the same Ambros group authors who authored the cited Lee, *et al.*, publication in 1993. This 2004 Lee, *et al.,* article is entitled "A Short History of a Short RNA", *Cell*, Vol. S116, S89-S92, January 23, 2004. In this article, Lee, *et al.,* discuss the work on the *lin-4S* and *lin-4L* transcripts on which they published in 1993. Their comments bear direct relevance to the present obviousness rejection, as the authors are not only people who had ordinary skill in the art, but were the leading experts in the field at the time, and thereafter.

The relevant section of this paper begins with the last full paragraph on page S90, and continues to the left-hand column of S91. The events leading to the discovery that the *lin-4S* short RNA was responsible for regulation of the *lin-14* gene based on its antisense sequence to the 3′-UTR thereof, as reported in the Lee, *et al.,* 1993 paper, is discussed. As noted at the top of S91, having recognized the antisense sequence, the Lee, *et al.,* group did attempt to discover whether other animals, including other nematodes, had similar sequences representing similar mechanisms. As reported at page S91, left-hand column, end of the first full paragraph:

> Candy (*i.e.*, Rosalind Lee) pressed forward with various hybridization strategies for cloning *lin-4* sequences from other animals, including other nematodes. **These attempts were unsuccessful, because, although we now know that even mammals have *lin-4*-related miRNAs (Lagos-Quintana, *et al.*, 2003), they are too divergent in sequence to have been identified by hybridization with *lin-4* probe.** (Emphasis added).

The relevance of this statement is apparent from the second document we are providing – a companion paper, Ruvkun, *et al.,* published back-to-back in the same issue of *Cell* as the above discussed Lee, *et al.,* 2004 paper. Ruvkun and his group have collaborated extensively with the Ambros group in untangling the relationship of the *lin-4* and *lin-14* genes in *C. elegans* (as noted, for example, in the Lee, *et al.,* 2004 paper in the sections cited above). This paper is entitled, "**The 20 Years it Took to Recognize the Importance of Tiny RNAs**", *Cell*, Vol. S116, S93-96, January 23, 2004. There are several statements made in this publication, (that is, in addition to the title of the publication itself) which have a direct bearing on the thesis underlying the obviousness rejection in this case:

At page S94, left-hand column, in the middle of the column, it is stated:

> To this day, *lin-14* homologs have been detected only in other nematodes, suggesting that the protein component of the *lin-4/lin-14* regulatory circuit is drifting fast or is an invention of the Nematoda.

which explains the relevance of this following quote at page S95, toward the top of the left-hand column:

> But the discovery of the world's first microRNA did not trigger a gold rush, not even by the Ambros or Ruvkun labs. First, the heterochronic pathway was a rather parochial object of study; **while the *lin-4* and *lin-14* stories were published in high profile journals, they were viewed as a novelty rather than a harbinger**. The antisense regulation was similar to some prokaryotic gene regulatory vignettes, if one ignored how incredibly small *lin-4* was (**and *lin-4* was four times smaller than any other noncoding regulatory RNA**). **Without homologs in other species, its generality did not emerge. We still did not suspect an extensive microRNA world even after Brenda Reinhart, Frank Slack, and others detected a second microRNA, *let-7* (Reinhart et al., 2000), because it emerged from genetic analysis of the same *C. elegans* heterochronic pathway; tiny RNAs could still have been inventions of this one pathway in this one species.** (Emphasis added).

These statements clearly eviscerate the basis for any obviousness thesis, and make it evident that the *lin-4/lin-14* discovery was, at the time of its discovery, considered to be almost an aberration even by those most skilled in the field, namely, those who discovered the *lin-4S* and *lin-4L* transcripts. These statements also confirm that no other such small RNA species was identified until the year 2000, subsequent to the priority date of the present application, and even then, when a second example was found, it was still considered likely to be a system that would not be present in any other species besides the nematodes (*i.e.*, it was thought that these "tiny RNAs could still have been inventions of this one pathway in this one species").

At page S95, right-hand column, first full paragraph, Ruvkun, *et al.*, state:

> An even deeper connection to RNAi started with numerological considerations (it cannot be called reasoning). When siRNAs of 22 nt, the same size as *lin-4* and *let-7*, were discovered by the Baulcombe and Tuschl groups in 1999 and 2001 (Hamilton and Baulcombe, 1999; Elbashir, *et al.*, 2001), Ruvkun noted that the number 22 (the number of letters in the Hebrew alphabet) is stressed in the Kabbalah, a Jewish mystical tradition celebrated in medieval Spain, alternative bookstores, and a number of helpful Web sites .... We began to explore the action of the RNAi machinery in miRNA maturation and activity.

This statement recognizes the significant contribution made by the inventors in the present application who published their discovery of the invariant correlation between detection of SRMs and PTGS in *Science* in 1999.

Finally, provided with this submission are reports of the award in 2008 by the Lasker foundation of a joint prize to Ruvkun, Ambros and Baulcombe for their respective roles in unraveling the role of short RNA molecules in the regulation of gene function as a general mechanism with far-reaching consequences for biological and medical research. It is generally acknowledged that the Lasker Prize is next in prestige only to the Nobel Prize itself, (which in 2006 was awarded to Drs. Fire and Mello for their discovery that long dsRNA is a trigger for inducing gene silencing).

The testimony of the authors of Lee, *et al.,* 1993 and their collaborators of the Ruvkun group undermines the obviousness rejection of the present claims, which do not reach detection of SRMs in nematodes, so that it cannot be sustained. It is respectfully requested that this ground for rejection be reconsidered and withdrawn.

4. The Rejection of Claims 32, 35, 36, 37, 41, 49 and 66 as Being Unpatentable Over Lee, *et al.*, and Further in View of Schena, *et al.* (1998, *Trends in Biotechnology*, 16:301-306)

The addition of Schena, *et al.,* is relevant only as to claim 41; the remaining claims have been distinguished from Lee above, and Schena does not cure any deficiencies of the document as to claims other than claim 41.

With respect to claim 41, Schena teaches the use of microarrays for determining expression levels. The possibility that the microarrays could contain a library of genes and reacted with tagged cDNA, for example, is discussed by Schena.

There is, however, no motivation to use the technique of Schena to determine a gene that is being silenced using the SRMs isolated according to the invention. This is because it is necessary to have made the present invention in order to recognize that it is the SRMs that need to be tagged. Absent that recognition, which is a result only of the present invention, there is no motivation to use the techniques of Schena.

Reconsideration and withdrawal of this ground for rejection is respectfully requested.

5. The Rejection of Claims 32-36, 49 and 66 as being Unpatentable over Noonberg, *et al.*

Claim 41 is free of this rejection.

Applicants do not entirely understand this rejection. The Office states that Noonberg teach gene regulation and gene therapy methods by generating antisense oligonucleotides of 20-50 nt in length. Noonberg is said to teach that the nucleotide generators can be used to deliver these to any type of eukaryotic cell although they do not mention nematodes explicitly.

The relevance of nematodes in the context of this case is not clear; the elected species for examination was mammals and in any event, nematodes are no longer included within the scope of the claims.

As to the rationale for the rejection in general, it is not clear why a teaching of a method to regulate genes by expressing an antisense oligonucleotide would have any bearing on a method to detect post-transcriptional gene silencing by assessing cellular extracts for short RNA molecules. Noonberg actually teaches away from such a suggestion by teaching, as noted above, in column 27, at lines 53, *et seq.*, that the transcript is "found predominantly in the nuclear fraction along with native U6. U6ON could not be detected to any significant extent in the cytoplasmic fraction." Since PTGS occurs after transcription in the cytoplasmic region, the teaching of Noonberg would suggest that the antisense RNA produces by its generator would not be associated with PTGS. Accordingly, this ground for rejection may be withdrawn.

6. Rejection of Claims 32-36, 41, 49 and 66 as Being Unpatentable Over Noonberg, *et al.*, in view of Schena, *et al.*

As discussed above, Noonberg neither anticipates nor provides a *prima facie* case for obviousness; indeed Noonberg teaches away from the claimed subject matter. The addition of Schena is relevant only to claim 41 and, of course, fails to remedy the defects in the claims from which claim 41 depends and therefore does not render claim 41 obvious either. Furthermore, there is no motivation to apply the method of Schena based on the art to the detection of PTGS and identification of the relevant genes since the prior art fails to suggest the relevance of short RNA molecules to this form of gene silencing. This rejection may be reconsidered and withdrawn.

Conclusion

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petitions for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 616292000110.

Respectfully submitted,

Dated: January 5, 2009 By: / Kate H. Murashige /

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